Introgression and Stabilization of *Erwinia* Tuber Soft Rot Resistance into Potato After Somatic Hybridization of *Solanum tuberosum* and *S. brevidens*

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ABSTRACT

Resistance to potato tuber soft rot caused by *Erwinia carotovora* was transferred from *Solanum brevidens* to the cultivated potato over the course of four backcross generations originating from a somatic hybrid. Soft rot reactions were determined via a tuber plug inoculation method developed during the course of these experiments. Soft rot resistance was highest in the somatic hybrid (only ca. 20% of tubers and plugs showed evidence of severe rotting) and lowest among progeny of control potato x potato crosses (ca. 80% of tuber plugs showed severe rot). Backcross generations involving somatic hybrids were intermediate in their reaction, and resistance stabilized to about 60% of tuber plugs showing severe rot in the BC_2 through the BC_4. Reciprocal crosses showed no difference in the inheritance of soft rot resistance, indicating that neither *S. brevidens* nor *S. tuberosum* donor cytoplasm had a significant effect on the expression of resistance. Crosses between BC_3 siblings where no *S. brevidens* genetic markers were detected but resistance was segregating demonstrated a dosage effect for soft rot resistance. We conclude that introgression of soft rot resistance has occurred and that at least one locus responsible for resistance in *S. brevidens* now resides in the *S. tuberosum* genome.

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ADDITIONAL KEY WORDS: Disease resistance, plant breeding, wild species, soft rot, *Erwinia* spp.

INTRODUCTION

Potato tuber soft rot can be a devastating post-harvest disease, particularly if mechanically damaged, insect-infested, or diseased tubers are placed in storage. Pre-emergence loss of rotten seed tubers and post-emergence losses from blackleg stem rot may also be severe at times. Each of these symptoms is caused by pectolytic species and subspecies within the bacterial genus *Erwinia* (Lyon 1989). These species are distributed worldwide in agricultural regions, but isolates differ in their pathogenicity.

Potato exhibits little genetic resistance to *Erwinia* infection; however, some varieties show a level of tolerance. Managing disease incidence and careful handling of tubers to minimize bruising prior to storage have been the best means to control soft rot in temperate climates, but without cooling facilities soft rots have precluded potato storage in tropical climates. An interplay between the ability of *Erwinia* to induce high levels of pectolytic enzymes (which in effect creates an anaerobic environment conducive to further bacterial growth) and the potato's ability to heal wounds and prevent further disease progression appears to determine whether extensive loss will occur (Lyon 1989). Thus, environment plays a critical role in disease expression.

The use of somatic hybridization in plant improvement is limited to those somatic hybrids that are fertile and that transmit some desirable trait to their progeny. *Erwinia* resistance was initially surmised when spring-planted seed tubers of somatic hybrids of potato and *S. brevidens* were recovered during the fall field harvest (Austin et al. 1988). Genetic analyses of the somatic hybrids and initial backcross derivatives with potato

have demonstrated the resistance to be heritable (Helgeson et al. 1993). Further backcrossing as well as additional inheritance data has reinforced the heritability of soft rot resistance (this report); however, the mechanisms and genetic factors responsible for resistance are still speculative.

MATERIALS AND METHODS

Plant Material

All somatic hybrid-derived materials used in these experiments came from a single somatic hybrid designated A206. This hybrid was obtained by fusion of protoplasts of *S. brevidens* PI 218228 (designated PS6A) and *S. tuberosum* PI 203900, (designated R4). Soft rot resistance in this and similar somatic hybrids have been described previously (Austin et al. 1988; Helgeson et al. 1993). Somatic hybrid A206 was crossed with *S. tuberosum* cv. Katahdin (KAT) to give a series of first backcross (BC_1) generation plants. One BC_1 plant (C75) was again crossed with KAT for the BC_2 population. One of these plants (C75-5) was crossed with *S. tuberosum* cv. Atlantic (ATL) to yield the BC_3 population. Plants with few or no *S. brevidens* specific genetic markers (RFLP or RAPD; McGrath et al. 1994, 1996) were selected from the BC_3 for either a fourth backcross to KAT (BC_4) or sibling crosses (BC_3F_1). A test population of R4 x KAT was constructed to examine inheritance of soft rot reaction in potato alone.

The number of plants tested for soft rot reaction in each generation is listed in Table 1. At least three tubers from each individual plant were tested per experiment. Tubers were harvested from field plots grown under agronomic conditions at Hancock, WI. Harvested tubers were stored dry at 4 C for at least three months and not longer than six months after harvest before testing.

Tuber Soft Rot Testing

Erwinia *carotovora* spp. *carotovora* strain ECC 394 was used for all inoculations. This strain was shown to be highly virulent (Austin et al. 1988). Analyses of the somatic hybrid, BC_2, BC_3, BC_3F_1, and BC_4 populations and a series of controls consisting of all parental materials and somatic hybrid A206 were conducted with a tuber plug assay, as follows. A single tuber plug 1.51 cm in diameter (#10 cork borer) was taken from each tuber transversely at the point of maximum girth. The tuber plug was trimmed to 2 cm in length such that the epidermis was removed and only the inner medullary tissue remained. Thus, influence of the epidermal layers on expression of resistance was minimized. Each plug was inoculated in the center of one end of the plug cylinder with 0.1 ml of bacterial suspension (10^8 CFU/ml) delivered with a 0.2 ml micropipet tip (i.e., Fischer Scientific, #21-197-8G) inserted 10 mm deep and left in place. Each inoculated plug was placed in a test tube (Kimax 25 mm x 150 mm; #45048-25150) and capped with an air-permeable, polypropylene closure (Kimble Kim-Kap; #73660-25). Each test tube contained a platform (e.g., a polypropylene microfuge tube) inside to prevent the plug from contacting any exudate from rotting plugs during the course of incubation. Incubation of the upright test tubes was at 24 C for 3 days in the dark, with humidity control provided solely by diffusion between ambient atmosphere and the internal atmosphere of the capped test tube. Plugs were removed and sliced longitu-

TABLE 1—*Descriptive statistics for the populations examined for soft rot resistance graphically represented in Figure 1 (SH = Somatic Hybrid). Higher values indicate a higher proportion of severely rotted tuber plugs in that population. Population code follows in each figure.*

-Cross	R4 x KAT	A206	A206 x KAT	C75 x KAT	75-5 x ATL	KAT x 277	277 x KAT	277 x 267	277 x 244
Population Code	A		B	C	D	E	F	G	H
Generation	Parent	SH	BC_1	BC_2	BC_3	BC_4	BC_4	BC_3 F_1	BC_3 F_1
Disease Reaction	S x S	R	R x S	R x S	R x S	S x R	R x S	R X S	R x R
N=	26	12	84	74	40	72	72	39	56
Mean	81.5	18.3	40.3	60.3	55.2	63.3	63.6	66.6	40.6
Std. Dev.	14.9	11.9	28.6	23.1	22.2	25.2	22.4	25.2	21.2
Minimum	49	5	0	12	16	9	10	12	8
Maximum	100	42	100	100	98	100	99	100	94
Median	86.5	16.0	40.5	65.0	53.6	66.5	67.0	69.0	39.5

dinally through the site of inoculation. Each plug was assigned two scores: a qualitative rating of R, I, or S (Resistant, Intermediate, Susceptible, respectively) and a semi-quantitative score based on the proportion of rotted tissue on the surface of the longitudinal slice. These observations were necessarily subjective; therefore, at least three independent workers scored the plugs in double blind replicated tests. The scores were highly reproducible among judges both within and between test populations ($r^2 > 90\%$). Statistical treatment of the data was explored using simple mean separation tools.

RESULTS AND DISCUSSION

We present data on the inheritance of soft rot resistance derived from *S. brevidens*. These data are not meant to be comprehensive nor conclusive, but simply suggest that, given the high experimental uncertainties, elucidating the inheritance of soft rot resistance may be possible. To do this we have taken a novel approach to screening large numbers of tubers for resistance reaction and have considered resistance a property of the population (i.e., a progeny test) and not of an individual plant (or tuber) within a population. In this work, rot scores from control lines (e.g., parental clone tubers) were considered more for comparative purposes rather than as benchmark resistance scores.

We developed and used a novel assay that allowed screening of a large number of tubers with limited resources. The primary requirement for this assay was that germplasm previously identified in whole tuber assays as highly resistant (e.g., A206) or highly susceptible (e.g., R4) (Helgeson et al. 1993) showed similar disease reaction in a tuber plug assay. While the assay was standardized in practice, a formal comparison of *Erwinia* soft rot methodologies was not our intention. This was because the level of resistance in the somatic hybrid A206 was much greater than the level of tolerance existing within potato that only the major differences were of interest here. We targeted the least resistant, internal medullary tissue of the tuber. Resistance from *S. brevidens* has been suggested to result from a structural modification of pectin (McMillan et al. 1993; Dorel et al. 1996). Alternatively, a regulatory gene may be involved. One of our most consistent observations has been that resistant lines appear to respond to infection faster than susceptible ones, and thereby "wall-off" the site of infection before the pathogen has a chance to multiply to detrimental levels.

Consistent and obvious differences were evident between susceptible tuber plugs (e.g., potato lines KAT, ATL, and R4) and resistant (e.g., somatic hybrid A206 and others) controls (Table 1). Occasionally one or more A206 tuber plugs would become completely soft and water-soaked, or the potato control would only show limited rot. Reasons for these inconsistencies may be due to minor variations in incubation temperature or innoculum load, but more likely they result from the uneven anatomy of the tuber tissue sampled. It was apparent that disease progressed more quickly through vascular tissues of the inoculated plug. These anatomical differences were not readily apparent in freshly cored tuber plugs.

In test populations, discrete classes were not observed and intermediate levels of plug rot were frequently encountered (Figure 1). Each of the populations showed an extreme range of disease reaction, with the exception of the potato parents population R4 x KAT. Similar distribution of scores across populations presented challenges in interpreting soft rot results. However, it was apparent from plotting the population means that these varied in an interpretable manner (Figure 2). That is, mean rot score for the potato parent cross (F_1), both scored as susceptible, was ca. 80%. In contrast, the mean score for A206, the somatic hybrid, among all tests (where it was always included as a control) was less than 20% (Table 1). The mean rating for a population of 15 independently derived somatic hybrids with the same parents as A206 was 19.1% (std. dev. = 11.4, range = 1 to 43, median = 17; data not shown). The somatic hybrids carried most or all of the two *S. brevidens* genomes from the diploid in addition to the four *S. tuberosum* genomes in the tetraploid recurrent parent. It should be noted that other somatic hybrids had better soft rot scores than A206; however, they were not as fertile as A206 and were thus more problematic to cross. Also, pollen fertility was high enough by the BC_3 that reciprocal crosses could be made effectively, and thus gauge the effect of cytoplasm on soft rot resistance.

A dosage effect may have been operating in the first backcross population, where population mean soft rot scores were ca 40% (Figure 2). With one exception, population mean soft rot scores were not significantly different between the BC_2 and subsequent generations (ca. 60%). This result suggests that no further segregation for *Erwinia* resistance occurred beyond the BC_2, and introgression had been effected by this generation. A dosage effect was particularly evident when two resistant BC_3 siblings were crossed, resulting in a reduction in population mean soft rot score to levels not significantly different than the BC_1 population (Figure 2; see also Figure 1H vs Figure 1G). It should be noted that no involvement of cytoplasmic factors was evident from reciprocal crosses in the BC_4 (Figures 1E and 1F).

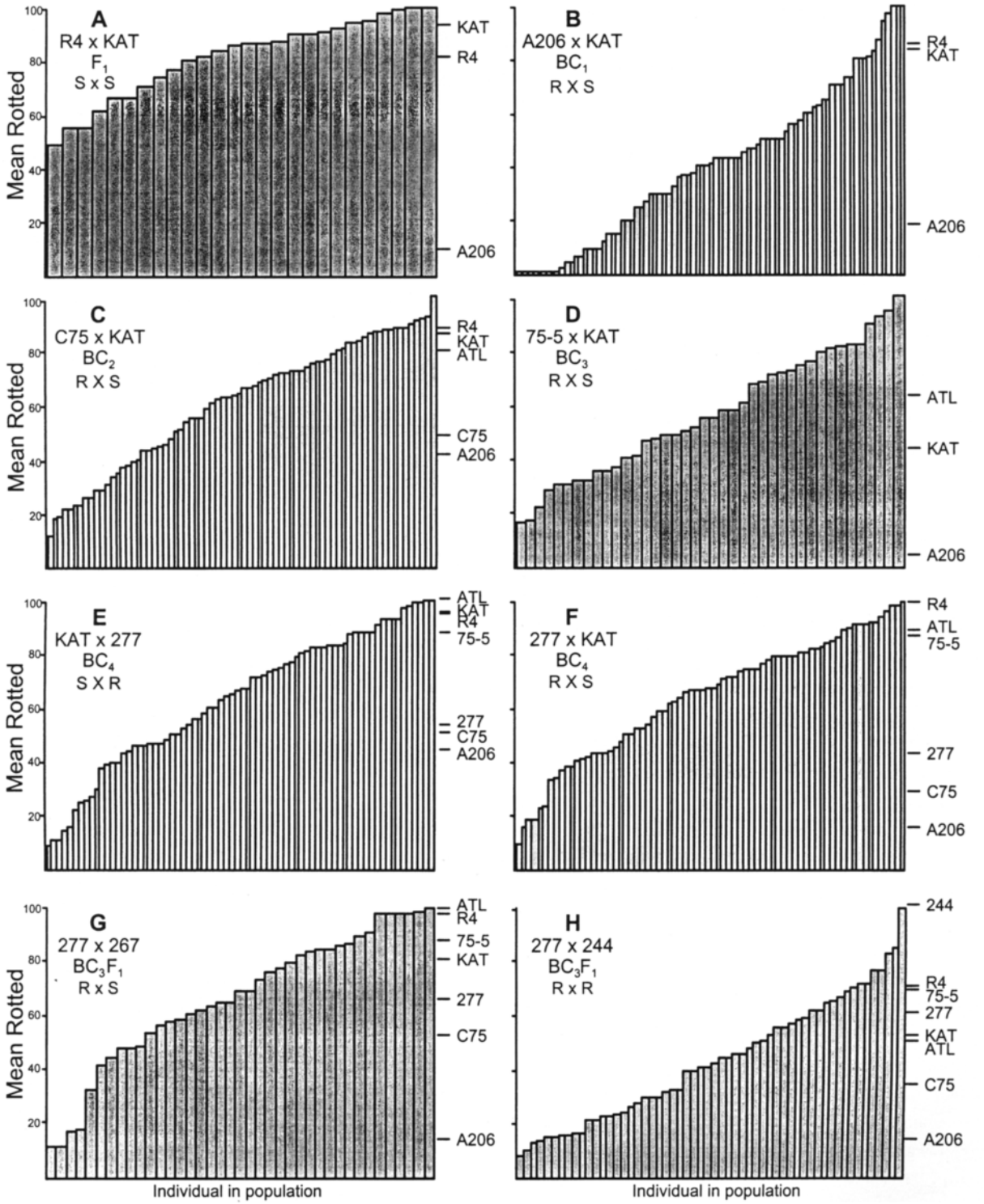

FIGURE 1.
Individual plant scores from each population as a mean of three or more tubers tested. Each bar represents a single line, and the parental values are indicated to the left of each panel.

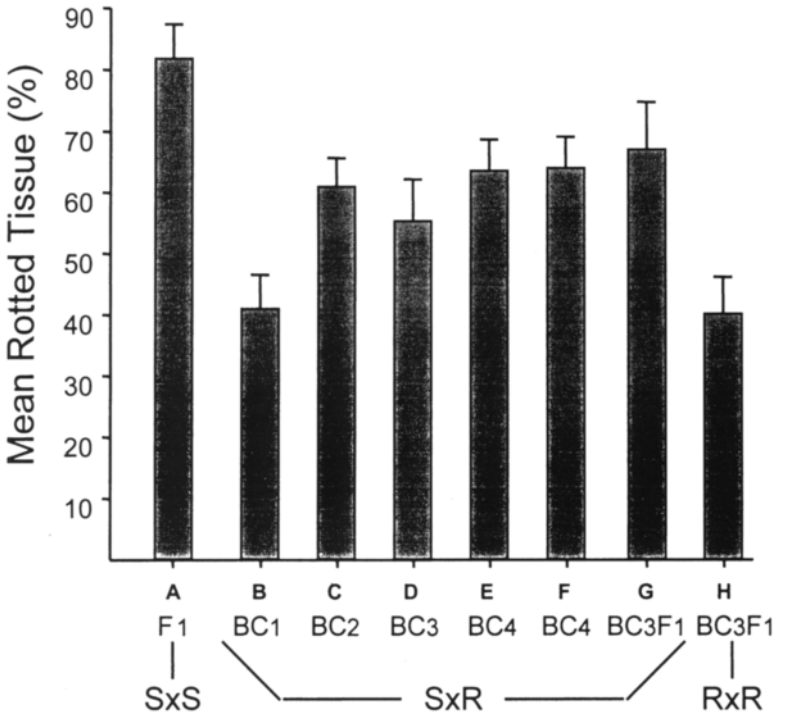

FIGURE 2.
Population mean soft rot score and 95% confidence interval for each population tested.

A great deal of molecular mapping data has been gathered on these populations (Williams et al. 1990, 1993; McGrath et al. 1994, 1996). The BC_2 somatic hybrid-derived plant used to generate the BC_3 population (e.g., 75-5) had a single *S. brevidens* synteny group for chromosome 8 and two other markers from independent synteny groups (McGrath et al. 1996). Plants in the BC_3 population had no detectable *S. brevidens* markers, although relatively few markers beyond those of chromosome 8 were tested, and had the euploid chromosome number 2n = 4x = 48 (data not shown). These results also suggest introgression of resistance had occurred by the BC_2 generation. It has not yet been possible to map these introgressed segments, but these would be critical for marker-assisted selection and map-based cloning approaches to determine gene function in the introgressed regions.

Monosomic and double monosomic addition lines for *S. brevidens* chromosomes were identified, each having all or most synteny group-specific markers for one or two *S. brevidens* synteny groups respectively. Seventeen BC_2 lines fit this criteria. Four lines with synteny groups 1, 3, and 4 were considered, as were two independent lines for each of synteny groups 7, 8, 9, 11, and 12, and one plant each for synteny groups 5 and 10. No progeny carried a single synteny group 2 or 6, although a related BC_2 population (e.g., C31 x KAT) did carry group 6 and was susceptible (data not shown). The only plants that were scored as resistant were those with synteny group 12. Thus, chromosome 12 appeared to carry a gene(s) for resistance to *Erwinia* tuber soft rot.

Allefs et al. (1995) examined inheritance of soft-rot and blackleg resistance among progeny derived from the same somatic hybrid used here, and were unable to deduce any clear inheritance patterns in the BC_1 and BC_2 generations. Our analyses have not conclusively demonstrated involvement of particular chromosomes, perhaps with the exception of chromosome 12 of *S. brevidens*. Preliminary statistical analyses of synteny group vs soft rot resistance implicate *S. brevidens* chromosomes 3, 5, and 9 as additional potential candidates harboring resistance genes (data not shown). The probable introgression of the soft rot resistance genes in early generations would have served to complicate genetic analyses in the BC_1 and BC_2 because in some progeny resistance would have been segregating with a potato chromosome(s) and in other progeny the original locus would have remained linked to *S. brevidens* chromosomes. Mapping should be performed in the BC_3 where *S. brevidens* chromosomes were not detected, leaving only the segregation of the introgressed *S. brevidens* chromosome segments as the source of the resistance trait.

Agronomic performance of all materials used here was collected. A brief comparison of the parental lines, and higher performing progeny lines, showed acceptable agronomic performance could be achieved from backcross derivatives of these somatic hybrids. For instance, breeding stock such as line 295 and line 297 showed a high proportion of commercial-sized tubers with few if any of the very small unmarketable tuber size class. Yields from these two lines (20.5 and 18.8 kg/10 plants, respectively) approached that of the commercial varieties KAT and ATL (20.7 and 17.3 kg/10 plants, respectively), and specific gravities were within the commercially acceptable range (1.103 for 297 and 1.087 for 295). It was concluded that introgression of soft-rot resistance did not affect the agronomic values of other essential traits in potato.

In summary, introgression of soft-rot resistance into potatoes has been accomplished, in part. Whether the levels of resistance observed here will be commercially useful remains to be

seen, but the resistance imparted here suggests that this range of reaction is not currently available to breeders. Resistance appears to be oligo-genic in *S. brevidens*, and it is likely that at least one gene, acting in a co-dominant fashion, has been transferred through somatic hybridization and subsequent backcrossing. No detrimental effects of resistance *per se* on agronomic performance were evident, and it is hoped that further characterization of this germplasm will lead to enhanced commercial varieties with increased tolerance to *Erwinia* soft-rots.

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We dedicate this paper to the late Thomas F. Uchytil.

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